

QuantiFERON[®]

The Whole Blood IFN- γ Test
An Aid to Detect *M. tuberculosis* Infection

Catalog Number: 0320 0201

PACKAGE INSERT

For *In Vitro* Diagnostic Use

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1. INTENDED USE

The QuantiFERON-TB test is an *in-vitro* diagnostic test intended as an aid in the detection of infection with *Mycobacterium tuberculosis*.

2. SUMMARY AND EXPLANATION OF THE TEST

Summary

The immune response to infection with *M. tuberculosis* is predominantly a Cell Mediated Immune (CMI) response. As part of this immune response, T-cells are sensitized to *M. tuberculosis* antigens. When stimulated with *M. tuberculosis* antigens such as tuberculin (a purified protein derivative from cultures of *M. tuberculosis*), memory T-cells, primarily CD4 but also CD8, produce the cytokine protein Interferon- γ (IFN- γ).

Memory T-cells also produce IFN- γ in response to other antigens when the immune system has been previously exposed to these antigens. Non-tuberculous Mycobacteria (NTM) share many antigenic components with *M. tuberculosis*, but also have antigenic components not shared with *M. tuberculosis*. Protein derivatives from other mycobacteria also elicit CMI responses. QuantiFERON-TB includes purified protein derivative (PPD) produced from *Mycobacterium avium* to assist with discriminating responses to *M. tuberculosis* from those due to reactivity to NTM. T-cells can also produce IFN- γ in response to mitogens, lectins such as phytohemagglutinin that nonspecifically bind to the T-cell receptor complex proteins and activate T-cells.

Explanation and Principles of the Assay

The QuantiFERON-TB assay detects CMI responses *in vitro* to tuberculosis infection by measuring IFN- γ harvested in plasma from whole blood incubated with tuberculin PPD, Mitogen, and *M. avian* PPD. The QuantiFERON-TB laboratory test is performed in two stages. First, four aliquots of heparinized whole blood are incubated with PPDs produced from *M. tuberculosis* (Human) and *M. avium* (Avian), as well as Mitogen and Nil control antigens.

After overnight incubation, plasma is removed and the amount of IFN- γ quantified by enzyme immunoassay (EIA). The QuantiFERON-TB EIA uses recombinant human IFN- γ standards, which have been assayed against a reference IFN- γ preparation (NIH Ref: Gxg01-902-535). Results for test samples are reported in International Units relative to this standard preparation.

The Mitogen-stimulated IFN- γ is used to normalize Human PPD responses. Only specimens with detectable amounts of IFN- γ from mitogen can be interpreted with the QuantiFERON-TB test. The Nil sample adjusts for background, heterophile antibody effects, or nonspecific IFN- γ in blood samples. Measurement of IFN- γ from the Avian PPD stimulated sample enables identification of specimens that may have predominant responses to non-tuberculous mycobacteria (e.g. *M. avium* complex) rather than *M. tuberculosis*. The comparison of IFN- γ levels detected in the four samples are used to determine the probability of infection with *M. tuberculosis* complex.

3. REAGENTS AND STORAGE

Components

1. Stimulation Antigens (*Contains 0.01% w/v Thimerosal*)

Nil Control	1 x 6mL
Human Tuberculin PPD	1 x 6mL
Avian Tuberculin PPD	1 x 6mL
Mitogen (phytohemagglutinin)	1 x 6mL

2. Microplate strips coated with anti-human IFN- γ murine monoclonal antibody
(2 x 96 well plates)

24 x 8 wells

3. Human recombinant IFN- γ Standards (*Contains recombinant human IFN- γ , bovine casein, 0.01% w/v Thimerosal*)

(1) Zero	1 x 1.5mL
(Actual IU/mL indicated on label) (2) Low	1 x 1.5mL
(3) Medium	1 x 1.5mL
(4) High	1 x 1.5mL

- | | |
|---|-----------|
| 4. Green Diluent (<i>Contains bovine casein, normal mouse serum</i>) | 1 x 15mL |
| 5. Conjugate 100X Concentrate (<i>Contains 0.01% w/v Thimerosal</i>)
(Murine monoclonal anti-human IFN- γ Horseradish Peroxidase) | 1 x 0.3mL |
| 6. Wash Buffer 20X Concentrate (<i>Contains 0.01% w/v Thimerosal</i>) | 1 x 100mL |
| 7. Enzyme Substrate Buffer (<i>Contains H₂O₂</i>) | 1 x 30mL |
| 8. Chromogen Solution (<i>Contains 3,3',5,5' Tetramethylbenzidine</i>) | 1 x 0.5ml |
| 9. Enzyme Stopping Solution (<i>Contains 0.5M H₂SO₄</i>) | 1 x 15mL |

Storage Instructions

Kit Reagents

- Store kit refrigerated at 2°C to 8°C.
- Always protect Chromogen from direct sunlight.

Reconstituted and Unused Reagents

- For instruction on how to reconstitute the reagents please see Preparation of Reagents in Section 6.
- Reconstituted **Standards** may be kept for up to 3 months if stored at 2°C to 8°C.
- Always note the date the **Standard** was reconstituted.
- Once reconstituted, unused **Conjugate 100X Concentrate** must be returned to storage at 2°C to 8°C immediately.
- Once reconstituted, **Conjugate 100X Concentrate** must be used within 3 months or discarded. Always note the date the **Conjugate 100X Concentrate** was reconstituted.
- Working strength **Conjugate** must be used within 30 minutes.
- Working strength **Wash Buffer** may be stored at room temperature for up to 2 weeks.
- Always use prepared **Enzyme Substrate** solution within 5 minutes of preparation.

4. WARNINGS AND PRECAUTIONS

Warnings

- QuantiFERON-TB has been evaluated for use with immunocompetent healthy adults with and without identified risk factors for tuberculosis infection.
 - QuantiFERON-TB has not been assessed in individuals with lymphocyte counts outside the normal range and the effect of lymphocyte count on reliability is unknown.
 - The performance of the QuantiFERON-TB test has not been evaluated in the following groups of individuals and it is not recommended for these population groups:
 1. Individuals who are immunocompromised such as those with HIV infection, AIDS, and transplant recipients.
 2. Persons with other clinical conditions that may compromise the immune system: diabetes, silicosis, chronic renal failure, hematological disorders (e.g., leukemia and lymphomas), and other specific malignancies (e.g., carcinoma of the head or neck and lung).
 3. Individuals who are immunosuppressed such as those taking immunosuppressive drugs (e.g. corticosteroids, methotrexate, azathioprine, chemotherapy).
 4. Individuals under the age of 17 years.
 5. Pregnant women.
-
- **For *in vitro* diagnostic use.**
 - **Handle human blood as if potentially infectious. Observe universal blood handling precautions (refer to NIH/CDC guidelines).**
 - **Handle Chromogen Solution with care. Avoid contact with skin and wear gloves.** This reagent contains dimethyl sulphoxide (DMSO), which is readily absorbed through the skin and may cause skin irritation. If Chromogen Solution comes into

contact with skin, wash the affected area immediately with copious quantities of water and seek medical attention.

- **Enzyme stopping solution is a strong acid.** Wipe spills up immediately and flush with water. If the stopping solution contacts the skin or eyes, flush with copious quantities of water and seek medical attention.
- **Thimerosal** is used as a preservative in some reagents. It may be toxic upon ingestion, inhalation or skin contact.
- **Green Diluent** contains normal mouse serum and casein which may trigger allergic responses; avoid contact with skin.
- Deviations from the package insert may yield erroneous results. Please read the instructions carefully before use.
- Use only Heparin as blood anticoagulant. Other anticoagulants interfere with the assay.
- Use of Costar Tissue culture plates is recommended; other culture plates have not been validated.
- Blood samples should be transported to the laboratory at ambient temperature ($22^{\circ}\text{C} \pm 5^{\circ}\text{C}$). Do not transport on ice or refrigerated.
- Blood must be incubated with stimulation antigens within 12 hours of collection; delay in incubation may cause false negative or indeterminate results.
- EIA substrate should NOT be prepared using polystyrene containers or pipettes. Polypropylene is recommended.
- A negative QuantiFERON-TB result does not preclude the possibility of TB infection. The specimen may have been obtained prior to development of cellular immune response, sufficient lymphocytes may not be present in the blood sample collected, or handling of the specimen may have affected lymphocyte function.
- A positive QuantiFERON-TB result should not be the sole or definitive basis for determining infection with *M. tuberculosis*. IFN- γ production may be due to cross reactivity with other mycobacterial antigens (e.g. BCG), or incorrect performance of the assay may cause false positive responses.
- Some specimens may not yield a measurable IFN- γ response to tuberculin. This may result in low IFN- γ readings and cause indeterminate QuantiFERON-TB test results.

Precautions

- Bring all components, except Conjugate 100X Concentrate, to room temperature (17°C to 27°C, 63°F to 81°F) before use.
- Store Conjugate 100X Concentrate at 2°C to 8°C at all times.
- Do not use kit if any reagent bottle shows signs of damage or leakage prior to use.
- Do not mix or use reagents from other QuantiFERON-TB kit batches.
- Do not use kit standards or conjugate after three months from reconstitution.
- Discard unused reagents and biological samples in accordance with local, state, and federal regulations.
- Do not use kit after the expiry date.
- Correct laboratory procedures should be adhered to at all times.

5. SPECIMEN HANDLING

Blood Collection

Collect 10mL of blood (**5mL minimum**) into a collection tube containing heparin as the anticoagulant. Gently mix by inverting the tube several times to dissolve the heparin, and transport to the laboratory at ambient temperature ($22^{\circ}\text{C} \pm 5^{\circ}\text{C}$). Blood should be incubated with stimulation antigens as soon as possible (as the percentage Human Response decreases with time) and culture with antigens must be initiated within 12 hours of blood collection. Up to 12 hours elapsed between blood collection and incubation with antigens during the clinical studies that established the sensitivity and specificity of the assay.

6. DIRECTIONS FOR USE

Time Required for Performing Assay

In order to obtain reliable results from the QuantiFERON-TB assay the operator needs to perform certain tasks within set times. Prior to use of the assay it is recommended that the operator plan each stage of the assay carefully to allow adequate time to perform each stage. The time required is estimated below:

Draw blood and initiate incubation	15 minutes
Incubation of blood samples with stimulating antigens	16–24 hours (overnight)
Human IFN- γ EIA stage	approx. 2 hours

Stage One – Incubation of Blood

Materials Provided

- QuantiFERON-TB kit stimulation antigens (Refer to Section 3).

Materials Required but not Provided

- Biohazard Cabinet Class II and a mechanical pipetting device.
- Sterile, 24 well tissue culture plates (6 patients/plate). Costar brand plates are recommended and use of plates from other manufacturers has not been validated.
- Sterile graduated 5 or 10mL pipettes (1 pipette/patient).
- 37°C humidified incubator (5% CO₂ optional).
- Calibrated variable-volume pipette capable of delivering 300–400 μ L with disposable tips.
- 1 mL microtubes with caps in 96 well format racks for plasma storage (20 patients/rack).
- QuantiFERON microplate shaker (Cellestis Cat. No. 08500201).
- Protective clothing for handling potentially infectious blood.

Stage One – Preparation of Reagents

STIMULATION ANTIGENS – Ready to use

























Contains 0.01% w/v Thimerosal

Stimulation antigens do not need to be brought to room temperature before use. Transfer the cardboard module containing the four stimulation antigens into the Biohazard cabinet. Use undiluted but mix thoroughly before use.

Stage One – Procedure

1. Blood samples must be evenly mixed before aliquoting. Use a roller-rocker or gently invert tubes 20 times *immediately prior to dispensing*.
2. Dispense four 1.0mL aliquots of heparinized whole blood from each patient into the wells of a 24 well tissue culture plate (see Figure 1 for recommended layout). Blood should be dispensed aseptically in a Biohazard cabinet using sterile pipettes to minimize the risk of contamination.
3. Prior to use, mix each stimulation antigen well. Holding the dropper bottle vertically, carefully add 3 drops of each antigen to the appropriate well(s).

FIGURE 1. Recommended layout for dispensing Blood and Stimulation Antigens into 24 Well Culture Plate

	Patient Sample Number					
	1	2	3	4	5	6
Nil Control (<i>gray cap</i>)						
Human PPD (<i>pink cap</i>)						
Avian PPD (<i>blue cap</i>)						
Mitogen (<i>purple cap</i>)						

4. Stimulation antigens must be mixed **THOROUGHLY** into the aliquoted blood using the QuantiFERON microplate shaker at the following setting; Waveform = 20, Amplitude = 9, Time = 1 to 2 minutes.

5. Incubate plates for 16–24 hours at 37°C in a humidified atmosphere.
 - **Avoid stacking plates during incubation.**
6. Carefully remove approximately 200–300µL of plasma from above the sedimented red cells using a variable volume pipette. Transfer the plasma into separate 1mL microtubes configured in the 96 well format outlined in Figure 2, Recommended Sample Layout. Label sample racks appropriately.
 - **Use a new pipette tip for each plasma sample.**
 - **Avoid harvesting blood cells with plasma. The assay will tolerate small quantities of cells, but if the harvested plasma sample is grossly contaminated with blood cells, centrifuge the sample to remove the cells.**
7. Plasmas can be stored at 2°C to 8°C for up to 14 days or for at least 3 months at or below –20°C. Microtubes should be sealed with an appropriate cap prior to storage to avoid evaporation.
 - **Plasmas may clot during extended storage. If clots are present refer to TROUBLE SHOOTING section.**

Stage Two – Human IFN- γ EIA

Materials Provided

- All QuantiFERON-TB kit components except stimulation antigens (Refer to Section 3).

Materials Required but not Provided

- Calibrated variable-volume pipettes capable of delivering 50µL, 300µL 500µL and 5–120µL with disposable tips.
- Multichannel pipette capable of delivering 50µL and 100µL with disposable tips.
- Variable speed vortex.
- Timer.
- Measuring cylinder-1L or 2L.
- Deionised or distilled water (EIA quality)-2L.

- QuantiFERON microplate shaker (Cellestis Cat No. 08500201).
- Microplate washer (optional).
- Microplate reader fitted with 450nm and 620nm (or 650nm) filters.
- Protective clothing for handling potential infectious material.
- Reservoirs (polypropylene).

Stage Two – Preparation of Reagents

1. PLATES – READY TO USE

Allow sealed plate(s) to equilibrate to room temperature for at least 60 minutes before opening. Remove strips that are not required from the frame, reseal in the foil pouch, and return to the refrigerator for storage until use.

Allow at least one strip for the QuantiFERON-TB standards and one strip for every two individuals being tested. After use, retain frame and lid for use with any remaining strips.

2. HUMAN IFN- γ STANDARDS

Contains 0.01% w/v Thimerosal

Reconstitute each vial with 1.5mL of deionised or distilled water *ensuring complete resolubilization*. Mix gently to minimize frothing.

3. CONJUGATE

Contains 0.01% w/v Thimerosal

Reconstitute freeze dried Conjugate 100X Concentrate with 0.3mL of deionised or distilled water. To *ensure complete resolubilization* of the Conjugate, mix thoroughly and gently to minimize frothing.

Working strength conjugate is prepared by diluting reconstituted Conjugate 100X Concentrate in Green Diluent as set out in Table 1 – Conjugate Preparation.

- **Mix thoroughly but gently to avoid frothing.**
- **Working strength conjugate should be used WITHIN 30 MINUTES OF PREPARATION.**
- **Return any unused Conjugate 100X Concentrate to 2°C to 8°C immediately after use.**
- **Use only Green Diluent as it contains normal mouse serum to compete out effects of heterophile antibodies in plasma samples.**

TABLE 1. CONJUGATE Preparation Table

NUMBER OF STRIPS	VOLUME OF CONJUGATE 100X CONCENTRATE	VOLUME OF GREEN DILUENT
1	5 μ L	0.5mL
2	10 μ L	1.0mL
3	15 μ L	1.5mL
4	20 μ L	2.0mL
5	25 μ L	2.5mL
6	30 μ L	3.0mL
7	35 μ L	3.5mL
8	40 μ L	4.0mL
9	45 μ L	4.5mL
10	50 μ L	5.0mL
11	55 μ L	5.5mL
12	60 μ L	6.0mL

4. WASH BUFFER*Contains 0.01% w/v Thimerosal*

Each plate (12 x 8 well strips) requires 1L of working strength wash buffer. Dilute one part Wash Buffer 20X Concentrate with 19 parts deionised or distilled water and mix thoroughly.

5. PREPARATION OF ENZYME SUBSTRATE SOLUTION

(Chromogen contains 3,3',5,5' Tetramethylbenzidine, Enzyme Substrate Buffer contains H₂O₂)

Prepare substrate just prior to use. Dilute appropriate volumes of Chromogen Solution 100X Concentrate in Enzyme Substrate Buffer as shown in Table 2, Substrate Preparation Table. Enzyme substrate solution must be completely mixed and should be colorless.

- Use Enzyme Substrate Solution within 5 minutes of preparation.
- Discard if blue coloration occurs.
- Substrate should NOT be prepared using polystyrene containers or pipettes. Polypropylene is recommended.

TABLE 2. SUBSTRATE Preparation Table

NUMBER OF STRIPS	VOLUME OF CHROMOGEN 100X CONCENTRATE	VOLUME OF ENZYME SUBSTRATE BUFFER
1	10 μ L	1mL
2	20 μ L	2mL
3	30 μ L	3mL
4	40 μ L	4mL
5	50 μ L	5mL
6	60 μ L	6mL
7	70 μ L	7mL
8	80 μ L	8mL
9	90 μ L	9mL
10	100 μ L	10mL
11	110 μ L	11mL
12	120 μ L	12mL

Stage Two – Procedure

1. All plasma samples and reagents, except for the Conjugate 100X Concentrate, must be brought to room temperature ($22^{\circ}\text{C} \pm 5^{\circ}\text{C}$) before use. Allow at least 60 minutes for equilibration.
2. Reconstitute freeze dried Standards and Conjugate 100X Concentrate.
3. Prior to assay, plasmas should be vortexed to ensure that IFN- γ is evenly distributed throughout the sample.
4. Dilute the required amount of Conjugate 100X Concentrate in Green Diluent according to the Conjugate Preparation Table (Table 1). Add 50 μ L of freshly prepared conjugate to the required EIA wells using a multichannel pipette.

5. Using a multichannel pipette, add 50µL of test plasma samples and standard samples to appropriate wells containing conjugate (see Figure 2). Standard samples should be added to each plate last and assayed at least in duplicate.

FIGURE 2. Recommended Sample Layout-Half/Whole Plate

A	1N	2N	3N	4N	5N	S1	S1	11N	12N	13N	14N	15N
B	1H	2H	3H	4H	5H	S2	S2	11H	12H	13H	14H	15H
C	1A	2A	3A	4A	5A	S3	S3	11A	12A	13A	14A	15A
D	1M	2M	3M	4M	5M	S4	S4	11M	12M	13M	14M	15M
E	6N	7N	8N	9N	10N	S1	S1	16N	17N	18N	19N	20N
F	6H	7H	8H	9H	10H	S2	S3	16H	17H	18H	19H	20H
G	6A	7A	8A	9A	10A	S3	S3	16A	17A	18A	19A	20A
H	6M	7M	8M	9M	10M	S4	S4	16M	17M	18M	19M	20M

S1–4 (Standards #1 to 4): 1N (Sample 1. Nil Control plasma); H (Human PPD plasma); A (Avian PPD plasma); M (Mitogen Control plasma)

- Vertical sample layout may be considered to maximize use of available EIA wells.
 - Standards should be assayed at least in duplicate.
6. Mix the conjugate and plasma samples/standards thoroughly using the QuantiFERON microplate shaker with Waveform set on twenty (20), Amplitude set on six (6) and Time set at one (1).
 7. Cover each plate with a lid and incubate at room temperature ($22^{\circ}\text{C} \pm 5^{\circ}\text{C}$) for 60 ± 5 minutes,
 - Plates should not be exposed to direct sunlight during incubation.
 - Deviation from the specified temperature range can lead to erroneous results.

8. Wash wells with 300-400 μ L of working strength wash buffer for at least 6 cycles at room temperature ($22^{\circ}\text{C} \pm 5^{\circ}\text{C}$). A fully automatic plate washer is recommended.
 - **Thorough washing is very important to the performance of the assay. Ensure each well is completely filled with wash buffer to the top of the well for each wash cycle.**
 - **Standard laboratory disinfectant should be added to the effluent reservoir and established procedures followed for the decontamination of potentially infectious material.**
9. After washing, dilute the required amount of Chromogen 100X Concentrate in Enzyme Substrate Buffer according to Table 2, Substrate Preparation Table.
10. Tap plates face down on an absorbent wipe to remove residual wash buffer. Add 100 μ L of freshly prepared substrate to each well and mix thoroughly using the QuantiFERON microplate shaker, adjusted to the settings described in Step 6.
 - **Commence incubation time as substrate is added to the first well(s).**
11. Cover each plate with a lid and incubate at room temperature ($22^{\circ}\text{C} \pm 5^{\circ}\text{C}$) for *precisely* 30 minutes.
 - **Plates should not be exposed to direct sunlight during incubation.**
 - **Deviation from the specified temperature range can lead to erroneous results.**
12. After 30 minutes add 50 μ L of Enzyme Stopping Solution to each well and mix by gentle agitation.
 - **Enzyme Stopping Solution should be added to wells in the same order and at the same speed as the substrate in step 10.**
13. Read the absorbance of each well within 5 minutes of terminating the reaction using a 450nm filter, with either a 620nm or 650nm reference filter. Absorbance values are used to calculate results.

7. DATA ANALYSIS AND TEST INTERPRETATION

Generation of Standard Curve

Determine the mean absorbance of the Human IFN- γ Standard replicates on each plate.

Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the known IFN- γ concentration of the standards in IU/mL (shown on each vial) on the x-axis. Draw a line of best fit through the points to generate a standard curve (linear regression). The curve is to be extended until it reaches the x-axis (beyond zero if necessary), but should not be extrapolated beyond the upper limit of the assays linear range (200 IU/mL).

To determine the IFN- γ concentration (IU/mL) for each of the test plasma samples, use the standard curve to read off the IFN- γ concentration (IU/mL) from the absorbance value of each sample. Negative IU values (those read off the curve to the left of the y-axis) should be used in all calculations.

These calculations can be generated automatically using standard software packages available with microplate readers, and standard spreadsheet or statistical software (such as Microsoft Excel). It is recommended that these packages be used to calculate the linear regression analysis, the % coefficient of variation (CV) for the standards, and the correlation coefficient (r) of the standard curve.

Quality Control of Test

The accuracy of test results is dependent on the EIA generating a linear curve. Therefore, results derived from the standards must be examined before test sample results can be interpreted.

For the EIA test to be valid:

- The correlation coefficient (r) calculated from the mean absorbances of the standards must be ≥ 0.98 .
- Replicate absorbances for the Zero Standard must not vary by more than 0.040 optical density units from their mean. If the Zero Standard is only tested in duplicate, the absorbances for the two values should not differ by more than 0.040 optical density units.

- The Low, Medium and High Standards must be within 15% of their individual mean absorbance values (% Coefficient of Variation (CV) < 15 %).
- The mean absorbance for the High Standard must be ≥ 0.700 .

If the above criteria are not met the run is invalid and must be repeated.

Each laboratory should determine appropriate types of control materials and frequency of testing in accordance with local, state, federal or other applicable accrediting organizations. External quality assessment and alternative validation procedures should be considered.

Calculation of Results

IFN- γ values are used to calculate % Human Response and % Avian Difference values for each patient as indicated below:

$$\% \text{ Human Response} = \frac{(H-N)}{(M-N)} \times 100$$

$$\% \text{ Avian Difference} = \frac{[(A-N)-(H-N)]}{(H-N)} \times 100$$

Where: N = IFN- γ (IU/mL) for Nil Control well
 H = IFN- γ (IU/mL) for Human PPD well
 A = IFN- γ (IU/mL) for Avian PPD well
 M = IFN- γ (IU/mL) for Mitogen Control well

Samples with absorbance values above the upper limit of the reader should be diluted and re-tested (see TROUBLE SHOOTING section).

Interpretation of Results

QuantiFERON-TB results are interpreted as follows:

M -N ¹ (IU/mL)	H -N ² (IU/mL)	% Human Response ³	% Avian Difference	Report	Interpretation
≥1.5	≥1.5	≥30%	≤10%	QuantiFERON-TB ≥30%	MTB infection likely
≥1.5	≥1.5	≥15% but <30%	≤10%	QuantiFERON-TB 15–30%	1. MTB infection not likely for low risk individuals; 2. MTB infection likely if risk identified.
≥1.5	All other response profiles			QuantiFERON-TB <15% or not significant	MTB infection NOT likely
<1.5	All other response profiles			QuantiFERON-TB INDETERMINATE	Result not obtained

1 M-N must be ≥ 1.5 IU/mL for a subject to have a valid QuantiFERON-TB result.

If M-N < 1.5 IU/mL the individual is deemed indeterminate for MTB infection regardless of their % Human Response and % Avian Difference results

2 H-N must be ≥ 1.5 IU/mL for a patient to be considered QuantiFERON-TB POSITIVE for MTB infection.

The lowest detectable response is 1.5 IU/ml. If H-N < 1.5 IU/mL the individual is deemed negative for MTB infection regardless of their % Human Response and % Avian Difference results

3 A 15% Human Response cut-off is used for individuals with identified TB exposure risk and a **30% cut-off** for people with no identified risk factors.

QuantiFERON-TB test results can only be interpreted from specimens capable of generating detectable levels of IFN-γ. The Mitogen Control generally elicits the greatest IFN-γ response of the 4 samples from each blood specimen.

Under most circumstances the Nil Control will not generate IFN-γ above 5 IU/ml – see section 9, Figure 3. The IFN-γ level of the Nil Control is considered background and is subtracted from the other results for that blood specimen.

The % Human Response calculation is used to normalize the variation in level of IFN-γ responsiveness between blood specimens. % Human Responses above designated cut-off values indicate significant reactivity, likely to have resulted from mycobacterial infection.

The % Avian Difference calculation is used to minimize cross-reactivity associated with non-tuberculous mycobacteria.

If an individual's % Human Response is greater than their appropriate cut-off but their % Avian Difference is >10% the test interpretation is QuantiFERON-TB negative for MTB infection. Reactivity to mycobacterial PPDs in such situations is not likely to be due to *M. tuberculosis* infection.

Two different cut-offs are used for the % Human Response value and are based on the individual's risk factors for *M. tuberculosis* exposure.

- **A ≥15% Human Response** indicates a likelihood of TB infection for individuals with recognized risk for TB exposure*.
- **A ≥30% Human Response** indicates a likelihood of TB infection in individuals with no identified risk factors for TB exposure.

*Exposure risks evaluated in the clinical studies include recent arrivals from high-prevalence countries, contacts of patients with tuberculosis, intravenous drug users, and persons who live, work, or volunteer on a regular basis in a homeless shelter, prison, drug rehabilitation unit, hospital, or nursing home.

The magnitude of the measured IFN- γ level cannot be correlated to stage or degree of infection, level of immune responsiveness, or likelihood for progression to active disease.

Physicians Instructions

A positive QuantiFERON-TB result does not necessarily indicate the presence or absence of active tuberculosis disease. Other diagnostic procedures, such as X-ray examination of the chest and microbiological examination of sputum should be used when TB disease is suspected.

The possibility should not be excluded that a positive QuantiFERON-TB result is due to prior BCG vaccination. BCG vaccinees included in clinical studies generally had a lower rate of positivity with QuantiFERON-TB than by skin testing (TST). The significance of this finding could not be established.

The intended use of the QuantiFERON-TB test is as an aid to the detection of TB infection. Individuals who are positive in the QuantiFERON-TB test have *in vitro* evidence of a cellular immune response to tuberculin PPD. The treatment options for a patient considered to have TB infection are at the discretion of the physician and should be based on all available laboratory results, patient history, and information.

For further information refer to the 'Clinicians Guide to QuantiFERON-TB' located on the Cellestis website: www.cellestis.com or contact Cellestis for a copy to be mailed, faxed or emailed.

Sample Calculations

If the following OD readings were obtained, the Mean OD and %CV would be:

	OD Readings	Mean OD	%CV
Zero Standards	0.017, 0.020	0.019	Not Applicable
Low Standards	0.213, 0.230	0.222	5.43
Medium Standards	1.012, 1.056	1.034	3.01
High Standards	1.956, 1.895	1.926	2.24

Based on the Standard curve, the calculated Correlation coefficient (r) = 0.999

Using the criteria specified in the Validation of Test Performance section the assay is determined to be valid.

Antigen OD responses

Human PPD	1.086	Mitogen	1.689
Avian PPD	0.126	Nil	0.025

The standard curve is used to convert the Antigen OD responses to International Units (IU/mL):

Human PPD	67.37 IU/mL	Mitogen	106.24 IU/mL
Avian PPD	5.49 IU/mL	Nil	-1.02 IU/mL

Calculations for determining QuantiFERON results:

$$\% \text{ Human Response} = \frac{(67.37 - -1.02)}{(106.24 - -1.02)} \times 100 = 63.8\%$$

$$\% \text{ Avian Difference} = \frac{[(5.49 - -1.02) - (67.37 - -1.02)]}{(67.37 - -1.02)} \times 100 = -90.5\%$$

In this case the subject's % Human Response is > 30% (and their % Avian Difference is < 10%). This result is interpreted as positive indicating the likelihood of infection with *M. tuberculosis*.

8. LIMITATIONS

False results may occur due to:

1. Incorrect technique
2. Use of any anticoagulant other than heparin
3. Incorrect transport of blood specimens
4. Excessive levels of circulating IFN- γ
5. Longer than 12 hours from blood specimen drawing
6. Incorrect incubation times or temperatures
7. Expired reagents or reconstituted components
8. Other deviations from the recommended test procedure

Effect of prior BCG vaccination on QuantiFERON-TB results has not been established. BCG vaccinees included in one clinical study were 6 times more likely to be QuantiFERON-TB negative and skin testing (TST) positive. The significance of this finding could not be established.

The performance characteristics of the QuantiFERON-TB test have not been extensively assessed in individuals greater than 50 years of age.

The effects of the TST on subsequent QuantiFERON-TB results has not been evaluated. Care should be taken when interpreting QuantiFERON-TB results in individuals who have received a tuberculin skin test (TST or Mantoux) within the last 12 months. QuantiFERON-TB results may be boosted or falsely positive following prior skin testing, and the effects of the TST on subsequent QuantiFERON-TB results has not been evaluated.

Heterophilic, e.g. human anti-mouse, antibodies in the serum or plasma of certain individuals are known to cause interference with many immunoassays.⁸ The effect of heterophile antibodies in the QuantiFERON-TB EIA is neutralized by the addition of normal mouse serum to the Green Diluent and the use of F(ab')₂ monoclonal antibody fragments as the IFN- γ capture antibody coated to the microtitre wells.⁵ Studies during development of the test showed that 12 of 201 (6%) plasma samples demonstrated high levels of heterophile antibody interference. The addition of 20% normal mouse serum to the Green Diluent abrogated this interference.⁵ It should be noted that subtraction of the IFN- γ value for the Nil sample from all other values prior to test interpretation provides an internal control for any possible residual heterophile antibody interference.

9. EXPECTED VALUES

The following range of % Human Response and % Avian Difference have been observed during the clinical trials conducted:

% Human Response: 0 to 200% with some values up to 2000%

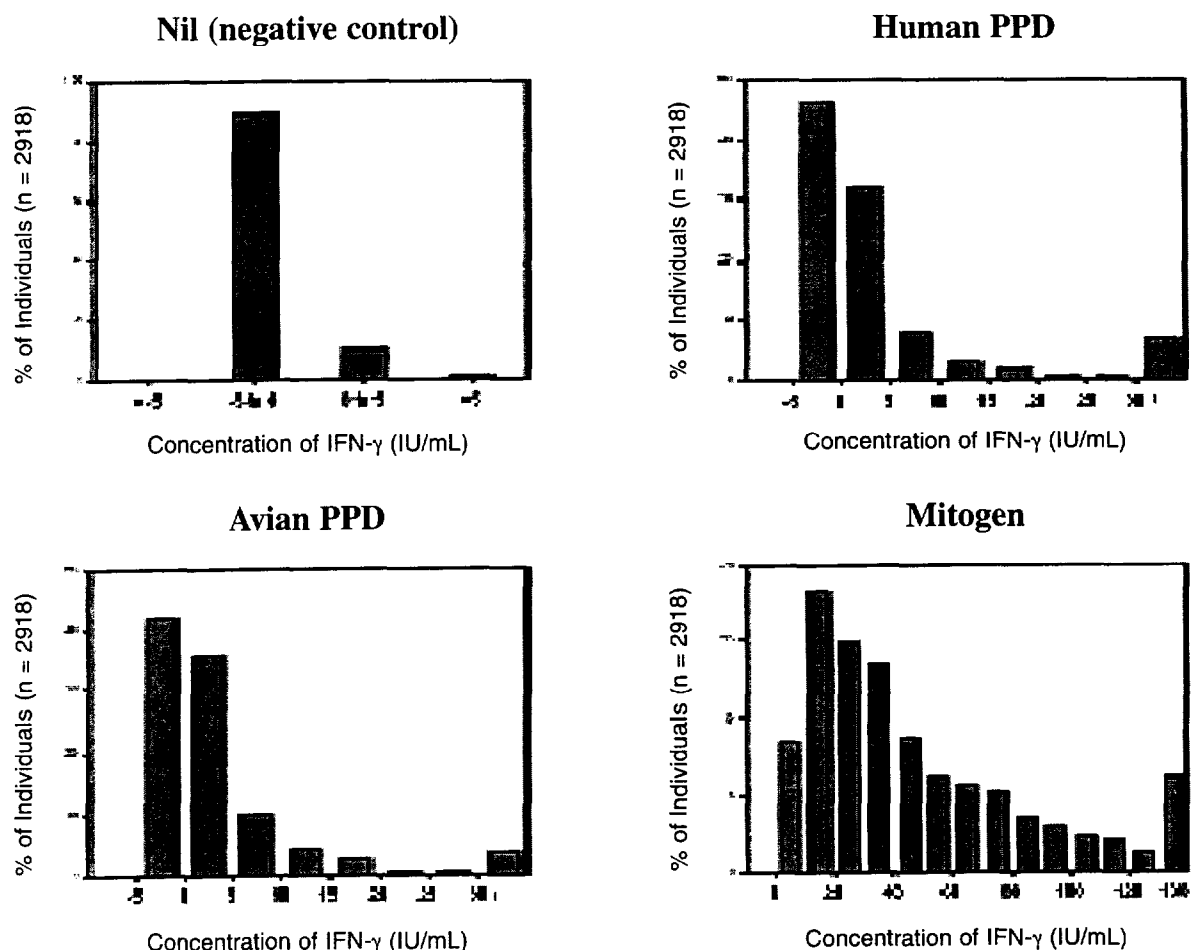
% Avian Difference: -300 to 300% with some values around 1000 and down to -2500%

For values obtained outside of these ranges it is recommended that the calculations be checked and repeated.

The distribution of the range for expected values in clinical trials for Nil, Human PPD, Avian PPD and Mitogen responses in IU/ml are presented in Figure 3.

FIGURE 3. Range of Expected Values for the QuantiFERON-TB Test

[Spread of Nil, Human PPD, Avian PPD and Mitogen responses in IU/ml from 2,918 subjects tested in clinical trials (see Section 10, Clinical Studies)]



10. PERFORMANCE CHARACTERISTICS

Clinical Studies

QuantiFERON-TB was evaluated at 6 clinical sites on specimens obtained in parallel to Tuberculin Skin testing. Five sites were part of a multicenter evaluation sponsored by the CDC⁷. These sites enrolled individuals with no identified risk (n=98 individuals requesting preemployment or preschool enrolment TST), and individuals with risk (n=944 asymptomatic subjects; see below for identified risk). TB suspects being evaluated for active TB who had less than 6 weeks of anti-TB therapy, and culture-confirmed TB subjects who completed treatment for TB within the prior 2 years were also enrolled. Subjects were excluded if they were <18 yrs, pregnant, HIV+, had a history of severe reaction to tuberculin, or were immunocompromised or had taken immunosuppressive drugs during the prior 3 months. Subjects ranged in age from 18 to 87 years (mean 39), 50.2% female, 72% were born in the US, and 38% were white, 35% were black, 13% were hispanic, 12% were asian and 2% were of other race. Of the total number of 1627 individuals recruited into the study, 5 had “Indeterminate” QuantiFERON-TB test results that were excluded from analyses. Four hundred and four additional subjects were excluded from analyses. No plasma samples required dilution and re-testing in the EIA.

The sixth site was a military recruiting center. TST was done at the time blood specimens were collected for the QuantiFERON-TB test. Subjects represented healthy, young (17–35 yrs), primarily male individuals (82.0%). Of the 1695 subjects, 232 were identified as having risk for LTBI (born or lived in a country with TB rate >10/100,000 or reported being a contact of a TB case).

The specificity of the QuantiFERON-TB test was estimated, using the 30% Human Response cut-off, by analyzing data from low risk subjects: 96/98 (98%; 95% CI 92–100%) and 1431/1463 (98%; 95% CI 97–99%) subjects from the CDC multicenter study and military recruits, respectively, were negative by the QuantiFERON-TB test. The majority of these individuals in these studies were under 30 years of age. For estimating specificity, all of these individuals were considered uninfected (see Figure 4).

In the absence of a reference standard for LTBI the QuantiFERON-TB test was compared to the Tuberculin Skin Test (TST) in subjects with no risk of *M. tuberculosis* infection and in those at risk of LTBI. The TST has been used for more than 50 years to

measure cell-mediated immunity resulting from *M. tuberculosis* infection, and uses PPD injected intradermally to elicit a delayed type hypersensitivity reaction. The diameter of the area of induration around the injection site indicates the response to PPD, and a stratified diagnostic cut-off is used; 10mm or greater indicates TB infection in those with known risk factors for infection, and 15mm for low risk individuals.

Figure 4. shows the agreement between QuantiFERON-TB results and the TST for people with no identified risk factors from both of the above studies.

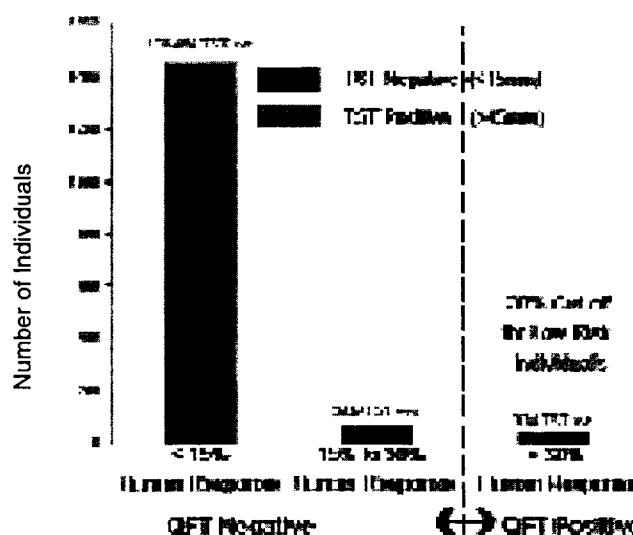
FIGURE 4. Comparison of results from QuantiFERON-TB and the TST for 1,561 people with no TB risk factors (CDC and Military Recruit studies).

	3 (0.2%)	16 (1.0%)
	31 (2.0%)	1511 (96.8%)

QuantiFERON-TB: (30% Human Response cut-off)

Tuberculin Skin Test: (15mm cut-off)

Kappa not applicable



The sensitivity of the QuantiFERON-TB test was estimated in the multicenter study conducted by the CDC. 44/54 individuals (81.5%; 95% CI 69–91%) with culture-confirmed TB disease were QuantiFERON-TB positive as compared to 49/54 (90.7%; 95% CI 80–97%) for the TST.

Figure 5(a) and 5(b) show QuantiFERON-TB test results compared to TST results for individuals at risk of latent TB infection in the CDC study and military study respectively. Agreement was 84% for the 944 individuals in the CDC study, and 83% in the military study, for those at risk of having LTBI.

FIGURE 5 (a). Comparison of results from QuantiFERON-TB and the TST for 944 people at risk of having LTBI (CDC study)

	145 (15.5%)	83 (8.4%)
	72 (7.5%)	644 (68.6%)

QuantiFERON-TB: (15% Human Response cut-off)

Tuberculin Skin Test: (using 10mm cut-off)

Kappa Coefficient of Agreement = 0.544,
McNemars $p = 0.422$

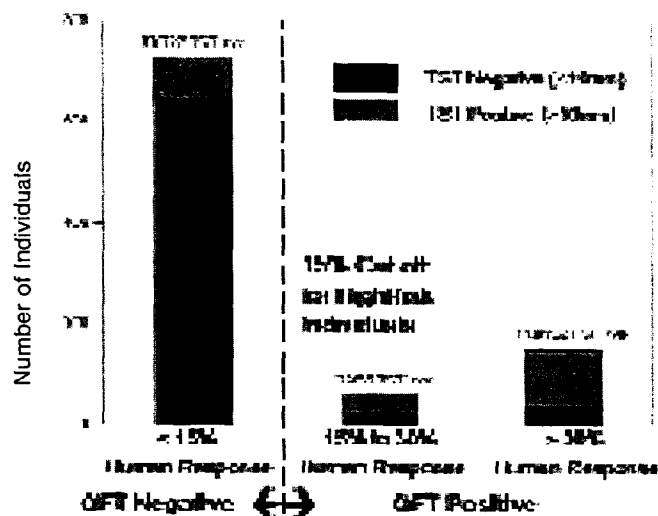


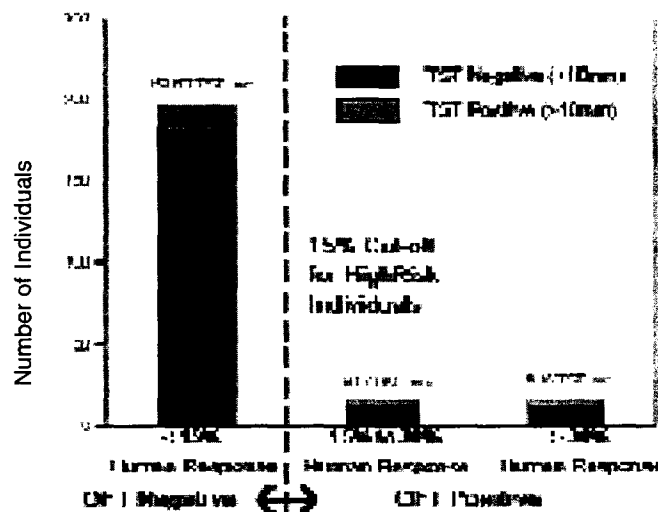
FIGURE 5 (b). Comparison of results from QuantiFERON-TB and the TST for 232 people at risk of having LTBI (Military Recruits)

	11 (4.7%)	15 (6.5%)
	24 (10.3%)	182 (78.5%)

QuantiFERON-TB: (15% Human Response cut-off)

Tuberculin Skin Test: (using 10mm cut-off)

Kappa Coefficient of Agreement = 0.266,
McNemars $p = 0.200$



The CDC results were analyzed for individuals with discordant results (positive in only one of the test systems). People with a history of BCG vaccination were more likely to be TST positive and QuantiFERON-TB negative. Males were more likely to be QuantiFERON-TB positive and TST negative. Thirteen (13) of the 83 TST positive/QuantiFERON-TB negative subjects were classified as QuantiFERON-TB negative due to predominant reactivity to PPD from *M. avium*. The factors which led to discordance in other individuals are unknown.

Assay Performance Characteristics

Studies have demonstrated that the % Human Response of blood specimens tested by QuantiFERON-TB decreases with the length of time blood is stored prior to incubation with stimulation antigens. For some QuantiFERON-TB positive individuals, this decrease may be significant (>50%) within the 12 hour recommended timeframe. For a small number of individuals (generally those with a % Human Response close to the relevant cut-off) the QuantiFERON-TB test result may alter from positive to negative, depending on the time of initiation of incubation post blood sample collection. Storage of blood samples for longer than the recommended 12 hours or outside of the quoted temperature range ($22^{\circ}\text{C} \pm 5^{\circ}\text{C}$) can lead to erroneous results.

The length of incubation of blood samples with the QuantiFERON-TB stimulation antigens was shown to be optimal between 16 and 24 hours. Additional studies with 4 blood donors showed no differences between blood incubated at 12 h or 24 h.

Studies with plasma samples from 2 donors demonstrated that natural IFN- γ is stable for at least 14 days when stored at 2°C to 8°C , or for at least 3 months when stored at or below -20°C . When re-testing samples, note that the test result may differ by $\pm 15\%$ (CV of the test); samples close to the diagnostic threshold may alter their diagnostic outcome. Plasmas spiked with recombinant IFN- γ have shown reductions in concentration over time of up to 50% when stored at either 2 to 8°C or -20°C , and recombinant IFN- γ is not recommended for establishing control standards.

The shelf life of the QuantiFERON-TB kit is 2 years from the date of manufacture when stored at 2°C to 8°C .

The linear range of the QuantiFERON-TB EIA for detecting IFN- γ (Sample value less Nil Control) is between zero and 200 IU/mL (correlation coefficient >0.99 , $n = 75 \times 4$).

The analytical sensitivity of the QuantiFERON-TB EIA is 1.5 IU/mL above the negative control (Nil) plasma sample for an individual. The Table below shows the accuracy of IFN- γ determinations, estimated using 16 sets of different plasma samples, “spiked” with known concentrations of IFN- γ ⁵.

IFN- γ (IU/mL) "spiked"	Mean IU/mL detected	% Coefficient of Variation	% Accuracy
150.0	154.6	3.0	103.1
75.0	86.7	2.7	115.6
37.5	43.5	4.4	115.9
18.8	20.8	5.6	110.7
9.4	9.8	8.2	104.5
4.7	4.0	9.5	85.3

11. TECHNICAL INFORMATION

Trouble Shooting

Difficulties that may be encountered in performing the assay include:

1. Clot formation in plasma samples that have been stored for an extended period of time. Clotted material can block multichannel pipette tips.
2. Very lipemic samples. Fatty deposits can block multichannel pipette tips.
3. Plasma samples with high levels of IFN- γ give optical density (OD) readings above the limit of the EIA reader. In such circumstances plasmas may require dilution so that they can be reassayed within the range of the reader.

How to deal with Clotted Plasma Samples

Firstly, centrifuge thawed samples in tube racks at 500g for 2 minutes to remove any plasma in the neck of the tubes. Carefully remove the tube cap band. *Care should be taken to avoid cross-contamination of samples.*

Mix each plasma tube by vortexing at moderate-high speed in a stop-start fashion, 3–5 times, with care. The purpose of this treatment is to facilitate sedimentation. For ease of handling, transfer mixed tubes to the same location in an empty rack. *Do not mix up tubes.*

Centrifuge the samples again at 500g for 2 minutes to sediment-clotted material. Care should be taken not to disturb pelleted material after centrifugation. Perform EIA.

How to deal with Plasmas that give Off-Scale OD Readings

One of the following procedures should be used if any or all of the PPD or Mitogen IFN- γ OD values are read by an EIA reader as off-scale.

1. Only the Mitogen stimulated IFN- γ plasma reads off-scale:

Calculate results giving the Mitogen plasma the highest possible IFN- γ value for that particular plate (taken from the standard curve). If the % Human Response is less than the appropriate cut-off (15% for people with identified TB risk factors, and 30% without risk factors) then presume that it is not clinically significant and report as calculated. If the corrected % Human Response is above 15%, then proceed to Step 2.

2. Any other plasma value reads off-scale:

Dilute all four plasmas by adding 1 volume of test plasma into 3 volumes of normal human serum (e.g. 50 μ L test plasma to 150 μ L of human serum), vortex at moderate-high speed in a stop-start fashion, 3-5 times, and repeat the EIA. The Nil Control value will correct for any IFN- γ contained in the normal human serum. Any source of normal human serum may be used.

The plate template section of the EIA reader software should be adjusted to reflect the change in sample concentration. Change the dilution factor to 4 for all repeat plasma samples. The report form should indicate this change and will automatically show the corrected level of IFN- γ in IU/mL for each diluted plasma.

If this software option is not available, multiply each re-assayed plasma IFN- γ value (in IU/mL) by 4 and proceed with subsequent calculations as detailed in the **CALCULATION OF RESULTS** section.

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13. TECHNICAL SERVICE

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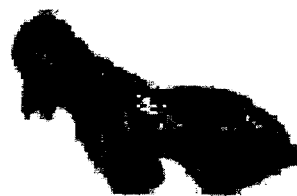
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14. ABBREVIATED TEST PROCEDURE

STAGE I - INCUBATION OF BLOOD

1. Draw 5–10mL blood into heparin tubes



2. Aliquot 1mL heparinized whole blood per antigen.



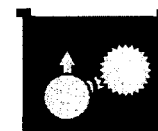
3. Add Human PPD, Avian PPD, Mitogen and Nil Control (3 drops per antigen).



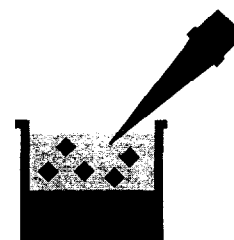
4. Thoroughly mix blood and antigens together.



5. Incubate overnight.

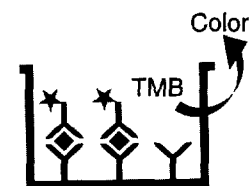
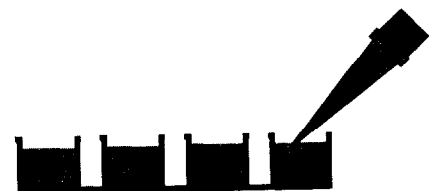
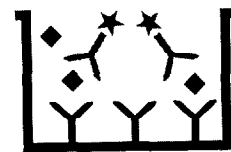
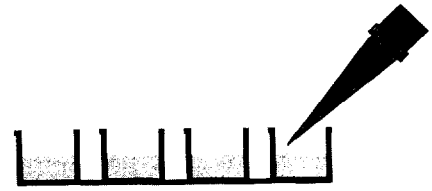


6. Harvest plasmas.



STAGE 2 - HUMAN IFN - γ EIA

1. Prepare Conjugate in Green Diluent and add 50 μ L to EIA wells.
2. Add 50 μ L test plasmas (simultaneously for each patient) and 50 μ L standards to wells.
3. Incubate for 60 minutes at room temperature.
4. Wash wells 6 times.
5. Prepare substrate and add 100 μ L to wells.
6. Incubate for 30 minutes at room temperature.
7. Add 50 μ L Stop Solution to wells.
8. Read results at 450 /620 (or 650) nm.





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